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- (71) Applicant(s)
SYNERGEN, INC.
- (72) Inventor(s)
DAVID F. CARMICHAEL; CHRISTOPHER G. SMITH; ROBERT C. THOMPSON
- (74) Attorney or Agent
GRIFFITH HACK & CO, GPO Box 1285K, MELBOURNE VIC 3001
- (57) Claim
1. A method for treating interleukin-1 mediated disease which comprises administering to a patient in need thereof a therapeutically effective amount of an interleukin-1 inhibitor.
9. The method of claim 4 wherein said IL-1ra is produced by recombinant DNA methods.

BAD ORIGINAL

FORM 1

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

I/We,

SYNERGEN, INC.
COLORADO

of

1885 33RD STREET
BOULDER
COLORADO 80301
USA

649245

heroby apply for the grant of a standard patent for an
invention entitled:

METHODS FOR TREATING INTERLEUKIN-1 MEDIATED DISEASES.

which is described in the accompanying complete specification

Details of basic application(s):

Number of basic application	Name of Convention country in which basic application was filed	Date of basic application
502745	US	
524210	US	02 APR 90
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		29 MAY 90

My/our address for service is care of GRIFFITH HACK & CO.,
Patent Attorneys, 601 St. Kilda Road, Melbourne 3004,
Victoria, Australia.

DATED this 20th day of March 1991

SYNERGEN, INC.

GRIFFITH HACK & CO



TO: The Commissioner of Patents.

PAD ORIGINAL

DECLARATION IN SUPPORT OF AN APPLICATION
FOR A PATENT

no. 73636/91

In support of an application made by

SYNERGEN, INC.

for a patent for an invention entitled

METHODS FOR TREATING INTERLEUKIN-1 MEDIATED DISEASES

of Gregory B. Abbott, Executive Vice President

of Synergen, Inc.

1855 - 33rd Street, Boulder, Colorado 80301, USA

do solemnly and sincerely declare as follows:

1. I am authorised by the above mentioned applicant to make this declaration on its behalf.

2. The name and address of each actual inventor of the invention is as follows: David F. CAMMICHIAI, 300 South Adams Drive, Louisville, Colorado 80027, U.S.A.; Christopher G. SMITH, 67 Baldwin Circle, Eldorado Springs, Colorado 80023, U.S.A.; Robert C. THOMPSON, 1320 Lough Street, Boulder, Colorado 80303, U.S.A.

3. The facts upon which the applicant is entitled to make this application are as follows:

The said applicant is the assignee of the said inventors

4. The basic applications, as defined by Section 141 of the Act, were made as follows:

in the U.S.A. on 2nd April 1990

in the names David F. CAMMICHIAI, Christopher G. SMITH & Robert C. THOMPSON

in the U.S.A. on 16th May 1990

in the name Robert C. THOMPSON

and in the U.S.A. on 19th May 1990

in the name Robert C. THOMPSON

5. The basic application referred to in the preceding paragraph was the first application made in the Convention country in relation to the subject of the application.

Declared at Boulder, Colorado

on 14th day of May 1991

Signed *Gregory B. Abbott*
Position Executive Vice President

GRIFFITH HACK & CO

ATTORNEYS FOR THE APPLICANT

BAD ORIGINAL

AUSTRALIA

PATENTS ACT 1952

COMPLETE SPECIFICATION

Form 10

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TO BE COMPLETED BY APPLICANT

Name of Applicant:

SYNERGEN, INC.

Address of Applicant: 1885 33RD STREET
BOULDER
COLORADO 80301
USA

Actual Inventor:

Address for Service: GRIFFITH HACK & CO.,
601 St. Kilda Road,
Melbourne, Victoria 3004,
Australia.

Complete Specification for the invention entitled:
METHODS FOR TREATING INTERLEUKIN-1 MEDIATED DISEASES.

The following statement is a full description of this invention
including the best method of performing it known to me:-

BAD ORIGINAL

METHODS FOR TREATING INTERLEUKIN-1 MEDIATED DISEASES

BACKGROUND OF THE INVENTION

The present invention describes methods for the treatment of a variety of diseases and medical conditions. The common element of the diseases and medical conditions that are suitable for treatment according to the methods described herein is an involvement of interleukin-1. This invention describes methods for the treatment of interleukin-1 mediated diseases and medical conditions.

Cytokines are extracellular proteins which modify the behavior of cells, particularly those cells that are in the immediate area of cytokine synthesis and release. One of the most potent inflammatory cytokines yet discovered and a cytokine which is thought to be a key mediator in many diseases and medical conditions is interleukin-1 (IL-1). Interleukin-1, which is manufactured, though not exclusively, by cells of the macrophage/monocyte lineage, may be produced in two forms, IL-1 alpha (IL-1a) and IL-1 beta (IL-1b).

A disease or medical condition is considered to be a "interleukin-1 mediated disease" if the spontaneous or experimental disease or medical condition is associated with elevated levels of IL-1 in bodily fluids or tissue or if cells or tissues taken from the body produce elevated levels of IL-1 in culture. In many cases, such interleukin-1 mediated diseases are also recognized by the following additional two conditions: (1) pathological findings associated with the disease or medical condition can be mimicked experimentally in animals by the administration of IL-1; and (2) the pathology induced in experimental animal models of the disease or medical condition can be inhibited or abolished by treatment with agents which inhibit the action of IL-1. In most "Interleukin-1 mediated diseases"

at least two of the three conditions are met, and in many "interleukin-1 mediated diseases" all three conditions are met. A list of diseases or medical conditions which are interleukin-1 mediated includes, but is not limited to, the following:

- 1) Arthritis
- 2) Inflammatory Bowel Disease
- 3) Septic Shock
- 4) Ischemic injury
- 5) Reperfusion injury
- 6) Osteoporosis
- 7) Asthma
- 8) Insulin diabetes
- 9) Myelogenous and other leukemias
- 10) Psoriasis
- 11) Cachexia/anorexia

Arthritis is a chronic joint disease which afflicts and disables, to varying degrees, millions of people worldwide. The disease is typically characterized at the microscopic level by the inflammation of synovial tissue and by a progressive degradation of the molecular components constituting the joint cartilage and bone. Continued inflammation and erosion of the joint frequently lead to considerable pain, swelling, and loss of function.

While the etiology of arthritis is poorly understood, considerable information has recently been gained regarding the molecular aspects of inflammation. This research has lead to the identification of certain cytokines, which are believed to figure prominently in the mediation of inflammation. The involvement of interleukin-1 in arthritis has been implicated by two distinct lines of evidence. First, increased levels of interleukin-1 and of the mRNA encoding it have been found in the synovial tissue and fluid of arthritic joints. References of interest include G. Buchan et al., Third Annual General Meeting of

the British Society for Rheumatology, London, England, November 19-21, 1988, PR. J. Rheumatol 25 (Supplemental 2) 1986; A. Fontana et al., Rheumatology Int., 2, pp. 49-53 (1982); and G. Duff et al., Monokines and Other Non-Lymphocytic Cytokines, M. Powanda et al., editors, pp. 387-392, 1988 Alan R. Liss, Inc.

Second, the administration of interleukin-1 to healthy joint tissue has been shown on numerous occasions to result in the erosion of cartilage and bone. In one experiment, intraarticular injections of IL-1 into rabbits were shown to cause cartilage destruction in vivo as described by E. Pettipher et al., Proc. Natl. Acad. Sci. USA, Vol. 83, pp. 8749-8753, November, 1986. In other studies, IL-1 was shown to cause the degradation of both cartilage and bone in tissue explants. Relevant references include J. Saklatavala et al., Development of Diseases of Cartilage and Bone Matrix, Alan R. Liss, Inc., pp. 291-298, and P. Stashenko et al., The American Association of Immunologists, Vol. 138, pp. 1464-1468, No. 5, March 1, 1987.

One generally accepted theory used to explain the causal link between IL-1 and inflammation is that IL-1 stimulates various cell types, such as fibroblasts and chondrocytes, to produce and secrete proinflammatory or degradative compounds, such as prostaglandin E2 and collagenase. Consequently, the present inventors postulated that substances that interfere with the activity of interleukin-1 would appear to make excellent candidates for use in the treatment of inflammatory diseases like arthritis.

Inflammatory bowel disease ("IBD") is a term used to describe both acute and chronic inflammatory conditions of the tissue of the intestinal tract and encompasses two generally distinct maladies known as Ulcerative Colitis and Crohn's disease. Ulcerative Colitis is a mucosal ulceration

of the colon. Crohn's disease, which is also referred to as Ileitis, Ileocolitis and Colitis, is a transmural inflammation that can be found throughout the general intestinal tract.

IBD is characterized by various histological features including transmural acute and chronic granulomatous inflammation with ulceration, crypt abscesses and marked fibrosis. Not all of these indications will be found in all IBD cases. Spontaneous reactivation, extraintestinal inflammation and anemia are often associated with IBD. Large joint arthritis is commonly found in patients suffering from Crohn's disease.

As is found in the molecular processes of the inflammation associated with arthritis, research has found that various cytokines appear to mediate aspects of IBD. In particular, IL-1 has been implicated as a mediating material in IBD. Again, two distinct lines of evidence lead to this conclusion.

Increased levels of IL-1 have been found in affected areas of intestines from patients with IBD. Tissues from patients with active Ulcerative Colitis showed IL-1 levels about 15 times the level found in control samples. Tissues with active Crohn's disease showed IL-1 levels about 6 times that of the control, and tissues with inactive Crohn's disease were about 3 times that of the control tissue samples. Sartor et al. Gastroenterology, 94, Pg. A399 (Abstract of Paper). See also Satsangi et al. Clin. Exp. Immunol., 67, Pp. 594-605 (1987); Rachmilewitz et al. Gastroenterology, 97, Pg. 326 (1989) (the bioassay used therein to determine IL-1 concentration levels is known to also unselectively detect IL-2, IL-4, IL-6 and IL-7).

The role of IL-1 in IBD has also been implicated by studies that have shown that the perfusion of rabbit colons with IL-1 induces the production of prostaglandin and thromboxane. Cominelli et al. Gastroenterology, 97, Pp.

1400-1405 (1989). This is consistent with the hypothesis described above, that IL-1 is linked to the inflammation of tissues due to its stimulation of the production of proinflammatory or degradative compounds. Thus, it is likely that systemic and local IL-1 production initiates or contributes to the inflammatory response in IBD, and plays an active role in the pathogenesis of the disease. The systemic production of IL-1 may also be responsible, in part, for the extraintestinal inflammation associated with Crohn's disease.

These results have led the inventors hereof to propose that substances that would interfere with the activity of interleukin-1 could be effective compounds for the treatment of IBD.

Septic shock is a condition associated with massive bacterial invasion. It is commonly believed that the shock is brought on, at least in part, by the presence of bacterial toxins (e.g., lipopolysaccharides). Septic shock is a relatively common cause of mortality in the hospital setting. At present there are few treatment options for patients suffering from septic shock, and the treatments available are generally supportive in nature.

Septic shock is characterized by various symptoms including a drop in mean arterial blood pressure (MAP), a decrease in cardiac output, tachycardia, tachypnea, lacticacidemia and leukopenia. Various cytokines, including interleukin-1, have been implicated in the mediation of septic shock, although the specific etiology of the disease is not fully understood.

That IL-1 may have a role in the mediation of septic shock has been suggested by two lines of evidence. One study has been conducted wherein the blood serum of children suffering from gram-negative septicemia was analyzed for IL-1 concentration. This study showed that elevated levels of IL-1 were found in 21% of the patients

examined. In addition, it was shown that IL-1 serum levels were significantly higher in patients who died than in the survivors. Girardin et al. New England J. of Medicine 319, pp. 397-400 (1988). See also, Cannon et al. Critical Care Medicine, p. S58 (Abstract) April, 1989 (increased IL-1 levels in patients suffering from sepsis syndrome).

It has also been shown that human IL-1 induces shock-like state in rabbits. A single bolus injection of human IL-1b brought about hypotension and several hemodynamic and hematological parameters characteristic of septic shock. For example, the MAP of IL-1 injected rabbits decreased by a minimum of 19.1%. Okusawa et al., J. Clin. Invest., 81, pp. 1162-1171 (1988).

These results have led the inventors hereof to propose that substances which interfere with the activity of interleukin-1 could be effective compounds for the treatment of septic shock.

Ischemic injury may occur to a tissue or organ whenever that tissue or organ is deprived of its normal blood flow. Further damage may occur when the flow of oxygenated blood is restored to that tissue. The extent and reversibility of the damage imparted depends, partly, on the severity of the original insult. It is possible, however, to mitigate the extent of tissue damage resulting from reperfusion by a variety of therapeutic interventions. Simpson PJ et al. In: Halliwell B. (ed) Oxygen Radicals and Tissue Injury, Brook Lodge Symp - Upjohn (1988).

Reperfusion injury is a well documented sequela to ischemic episodes in the heart, gut, kidney, liver and other organs. Simpson PJ et al. In: Halliwell B. (ed) Oxygen Radicals and Tissue Injury, Brook Lodge Symp - Upjohn (1988); Herman B. et al. FASEB J. 2:1460151 (1988); McDougal WS. The J. of Urology, 140:1325-1330 (1988); Finn WF. Kidney Int., 7:171-182 (1990); Schrier RW. Klin Wochenschr, 66:800-807 (1988); and Winchel RJ. Transplantation 48:393-396

(1989). The exact pathogenesis of reperfusion injury may vary depending on the tissue affected. In the heart, for instance, reperfusion injury is accompanied by a dramatic influx of neutrophils, and these cells are thought to play a major role affecting the reperfusion damage. Lucchesi BR et al. Ann Rev Pharmacol Toxicol 26:201-224 (1988). Renal ischemia and reperfusion injury, on the other hand, appear to involve an increase in tubular cell membrane permeability, increased levels of intracellular calcium, altered mitochondrial respiratory function, and the generation of free radicals. In the kidney, the role of extravasating neutrophils in affecting the reperfusion injury is less certain. McDougal WS. The J. Urology, 140:1325-1330 (1988); Finn WF. Kidney Int., 37:171-182 (1990); Schrier RW. Klin Wochenschr, 66:800-807 (1988); and Winchel RJ. Transplantation 48:393-396 (1989).

Despite the differences in cellular participation during ischemia and reperfusion injury, there may be similarities in the underlying mechanism. Interleukin-1 is recognized as an early stage mediator of organ injury, and may be generated by resident or newly infiltrated inflammatory cells giving rise to organ specific tissue pathology. In such an instance, the ability to inhibit the biological activity of IL-1 would represent a novel therapeutic intervention aimed at limiting the extent of tissue damage.

This information has led the inventors hereof to propose that substances which interfere with the activity of interleukin-1 could be effective compounds for the minimization of ischemia and reperfusion injury.

To date, an effective, yet selective, inhibitor of IL-1 has not been available in sufficient quantities or purity to prove that IL-1 is a target for pharmacological intervention in the treatment of arthritis, IBD, septic shock, ischemia injury or reperfusion injury and for use as

a therapeutic agent in the treatment of inflammation.

Despite the prior art, the present inventors have identified a class of compounds, referred to herein as interleukin-1 inhibitors, that prevent and treat interleukin-1 mediated diseases. Moreover the present therapeutic interventions may be practiced without unacceptable compromise of normal physiological processes (e.g. immuno competency) which are essential to the patients well being.

In currently pending U.S. Patent Application Serial No. 07/506,522, filed April 6, 1990 specifically incorporated herein by reference, a preferred class of naturally occurring, proteinaceous interleukin-1 inhibitors and a method for manufacturing a substantial quantity of the same with a high degree of purity are described. In particular, the aforementioned application describes in detail three such interleukin-1 inhibitors which are interleukin-1 receptor antagonists (IL-1ra's), namely, IL-1 α (IL-1 α), IL-1 β IL-1 β , and IL-1 γ . These IL-1ra's will be referred to as IL-1raa, IL-1rab and IL-1rax, respectively, in this application.

SUMMARY OF THE INVENTION

The present invention describes a method for the treatment of interleukin-1 mediated diseases by administering to patients in need thereof a therapeutic agent.

An object of the present invention is to provide methods for treating interleukin-1 mediated arthritis, interleukin-1 mediated inflammatory bowel disease ("IBD"), interleukin-1 mediated septic shock, interleukin-1 mediated ischemia injury and interleukin-1 mediated reperfusion injury by administering to patients in need thereof a therapeutic agent.

Additional objects and advantages of this

invention will be set forth in part in the description which follows and in part will be obvious from the description or may be learned from practice of the invention. The objects and advantages may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

To achieve the objects and in accordance with the purposes of the present invention, methods are disclosed for treating interleukin-1 mediated diseases, including interleukin-1 mediated arthritis, interleukin-1 mediated inflammatory bowel disease, interleukin-1 mediated septic shock, interleukin-1 mediated ischemia injury and interleukin-1 mediated reperfusion injury. These methods comprise administering to those patients in need thereof a therapeutically effective amount of an interleukin-1 inhibitor.

Preferred interleukin-1 inhibitors of the present invention are proteins and, more particularly, are naturally-occurring proteins. The naturally-occurring proteins are preferred because they pose a relatively low risk of producing unforeseen side effects in patients treated therewith.

A preferred class of interleukin-1 inhibitors are the human proteins which naturally serve as interleukin-1 receptor antagonists (IL-1ra's). Preferably, those IL-1ra's which are preferred in the practice of the present invention are selected from the group consisting of IL-1raa, IL-1raB, IL-1rax, or the N-terminal methionyl derivatives of IL-1rax. Also preferred are proteins which have been modified for example by the addition of polyethylene glycol (PEG) or any other repeat polymer to increase their circulating half-life and/or to decrease their immunogenicity.

While the production of IL-1ra may be achieved by extraction from naturally available sources, such as by isolation from the conditioned medium of cultured human

monocytes, a preferred method of IL-1ra production is by recombinant DNA technology. Recombinant DNA technology is preferred in part because it is capable of producing comparatively higher amounts of IL-1ra at greater activities.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the release of glycosaminoglycans (GAG) from bovine nasal cartilage in response to increasing amounts of IL-1B;

FIG. 2 depicts the inhibitory effect of increasing amounts of IL-1ra on the IL-1B-induced release of GAG from bovine nasal cartilage;

FIGS. 3 and 4 depict the inhibitory effect of IL-1ra on the pathogenesis of type II collagen-induced arthritis in mice;

FIG. 5 depicts the inhibitory effect of IL-1ra on SCW reactivation of SCW-induced arthritis in rats.

FIG. 6 depicts the effects of IL-1ra treatment on various indications of interleukin-1 mediated IBD.

FIG. 7 depicts the effects of IL-1ra on PG-APS reactivation of joint inflammation in conjunction with indomethacin.

FIG. 8 depicts the effects of IL-1ra on survival rate in rabbits with endotoxin-induced shock.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Reference will now be made in detail to the presently preferred embodiments of the invention, which, together with the following examples, serve to explain the principles of the invention.

As noted above, the present invention relates to

methods for treating interleukin-1 mediated diseases including interleukin-1 mediated arthritis, interleukin-1 mediated inflammatory bowel disease, interleukin-1 mediated septic shock, interleukin-1 mediated ischemia injury, and interleukin-1 mediated reperfusion injury in patients suffering therefrom. This method comprises the administration of a therapeutically effective amount of an interleukin-1 inhibitor. In one embodiment, the preferred interleukin-1 inhibitors of the present invention are naturally-occurring proteins that serve as IL-1 receptor antagonists (IL-1ra's).

A disease or medical condition is considered to be a "interleukin-1 mediated disease" if the spontaneous or experimental disease or medical condition is associated with elevated levels of IL-1 in bodily fluids or tissue or if cells or tissues taken from the body produce elevated levels of IL-1 in culture. In many cases, such interleukin-1 mediated diseases are also recognized by the following additional two conditions: (1) pathological findings associated with the disease or medical condition can be mimicked experimentally in animals by the administration of IL-1; and (2) the pathology induced in experimental animal models of the disease or medical condition can be inhibited or abolished by treatment with agents which inhibit the action of IL-1. In most "Interleukin-1 mediated diseases" at least two of the three conditions are met, and in many "interleukin-1 mediated diseases" all three conditions are met. A list of diseases or medical conditions which are interleukin-1 mediated includes, but is not limited to, the following:

- 1) Arthritis
- 2) Inflammatory Bowel Disease
- 3) Septic Shock
- 4) Ischemic injury
- 5) Reperfusion injury

- 6) Osteoporosis
- 7) Asthma
- 8) Insulin diabetes
- 9) Myelogenous and other leukemias
- 10) Psoriasis
- 11) Cachexia/anorexia

The naturally-occurring proteins are preferred in part because they pose a comparatively low risk of producing unforeseen and undesirable physiological side effects in patients treated therewith.

For purposes of the specification and claims, a protein is deemed to be "naturally-occurring" if it or a substantially equivalent protein can be found to exist normally in healthy humans. "Naturally-occurring" proteins may be obtained by recombinant DNA methods as well as by isolation from cells which ordinarily produce them. "Naturally-occurring" encompasses proteins that contain an N-terminal methionyl group as a consequence of expression in *E. Coli*.

"Substantially equivalent" as used throughout the specification and claims is defined to mean possessing a very high degree of amino acid residue homology (See generally M. Dayhoff, Atlas of Protein Sequence and Structure, Vol. 5, p. 124 (1972), National Biochemical Research Foundation, Washington, D.C., specifically incorporated herein by reference) as well as possessing comparable biological activity.

Particularly preferred IL-1ra's of the present invention are the naturally-occurring proteins that exist in vivo as regulators of interleukin-1 that have previously been described in a currently pending United States patent application. This application is U.S. Patent Application Serial No. 07/266,531, filed November 3, 1989, by Hannum et al., which is entitled "Interleukin-1 Inhibitors." This U.S. patent application is specifically incorporated herein

by reference.

Three preferred forms of IL-1ra, each being derived from the same DNA coding sequence, were disclosed and described in the aforementioned Hannum et al. application. The first of these, IL-1raa, was characterized as behaving as a 22-23 kD molecule on SDS-PAGE with an approximate isoelectric point of 4.8, eluting from a Mono Q FPLC column at around 52 mM NaCl in Tris buffer, pH 7.6. The second, IL-1raB, was characterized as behaving as a 22-23 kD protein, p.I=4.8, eluting from a Mono Q column at 60 mM NaCl. The third, IL-1rax, was characterized as behaving as a 20 kD protein, eluting from a Mono Q column at 48 mM NaCl. All three of the Hannum et al. interleukin-1 inhibitors were shown to possess similar functional and immunological activities. The present invention also includes modified IL-1ra's. In one embodiment, the IL-1ra is modified by attachment of one or more polyethylene glycol (PEG) or other repeating polymeric moieties. In another embodiment, the IL-1ra contains an N-terminal methionyl group as a consequence of expression in *E. coli*.

Methods for producing the Hannum et al. inhibitors are also disclosed in the above mentioned application. One disclosed method consisted of isolating the inhibitors from human monocytes (where they are naturally produced). A second disclosed method involved isolating the gene responsible for coding the inhibitors, cloning the gene in suitable vectors and cell types, expressing the gene to produce the inhibitors and harvesting the inhibitors. The latter method, which is exemplary of recombinant DNA methods in general, is a preferred method of the present invention. Recombinant DNA methods are preferred in part because they are capable of achieving comparatively higher amounts at greater purities.

Additional interleukin-1 inhibitors include compounds capable of specifically preventing activation of

cellular receptors to IL-1. Such compounds include IL-1 binding proteins such as soluble receptors and monoclonal antibodies. Such compounds also include receptor antagonists and monoclonal antibodies to the receptors.

A second class of IL-1ra's include the compounds and proteins which block in vivo synthesis and/or extracellular release of IL-1. Such compounds include agents which affect transcription of IL-1 genes or processing IL-1 preproteins. Under certain conditions, the IL-1ra will block IL-1 induced IL-1 production.

Preferably, the above described IL-1ra's are produced by the aforementioned method in "substantially pure" form. By "substantially pure" it is meant that the inhibitor, in an unmodified form, has a comparatively high specific activity, preferably in the range of approximately 150,000-500,000 receptor units/mg as defined by Hannum et al. in Nature 343: 336-340 (1990) and Eisenberg et al. in Nature 343: 341-346 (1990), both of which are specifically incorporated herein by reference. It is to be recognized, however, that derivatives of IL-1ra may have different specific activities. In a preferred embodiment of the present invention, a therapeutic composition comprising at least one of IL-1raa, IL-1raB, and IL-1rax is administered in an effective amount to patients suffering from interleukin-1 mediated diseases.

Because it is possible that the inhibitory function of the preferred inhibitors is imparted by one or more discrete and separable portions, it is also envisioned that the method of the present invention could be practiced by administering a therapeutic composition whose active ingredient consists of that portion (or those portions) of an inhibitor which controls (or control) interleukin-1 inhibition.

The therapeutic composition of the present invention is preferably administered parenterally by

injection, although other effective administration forms, such as intraarticular injection, inhalant mists, orally active formulations, or suppositories, are also envisioned. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment it is envisioned that the carrier and the IL-1ra constitute a physiologically-compatible, slow-release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the IL-1ra. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or requiring reconstitution immediately prior to administration. The preferred storage of such formulations is at temperatures at least as low as 4°C and preferably at -70°C. It is also preferred that such formulations containing IL-1ra are stored and administered at or near physiological pH. It is presently believed that storage and administration in a formulation at a high pH (i.e. greater than 8) or at a low pH (i.e. less than 5) is undesirable.

Preferably, the manner of administering the formulations containing IL-1ra is via an intraarticular,

subcutaneous or intramuscular route. Preferably, the manner of administering the formulations containing IL-1ra is via intra-articular, subcutaneous, intramuscular or intravenous injection, suppositories, enema, inhaled aerosol, or oral or topical routes. To achieve and maintain the desired dose of IL-1ra, repeated subcutaneous or intramuscular injections may be administered. Both of these methods are intended to create a preselected concentration range of IL-1ra in the patient's blood stream. It is believed that the maintenance of circulating concentrations of IL-1ra of less than 0.01 ng per ml of plasma may not be an effective composition while the prolonged maintenance of circulating levels in excess of 100 ug per ml may have undesirable side effects.

A preferred dosage range for the treatment of interleukin-1 mediated arthritis is between 1 and 100 ng/ml. Accordingly, it is preferred that, initially, doses are administered to bring the circulating levels of IL-1ra above 10 ng per ml of plasma and that, thereafter, doses are administered at a suitable frequency to keep the circulating level of IL-1ra at or above approximately 10 ng per ml of plasma. The frequency of dosing will depend on pharmacokinetic parameters of the IL-1ra in the formulation used.

A preferred dosage range for the treatment of interleukin-1 mediated IBD is between about .5-50 mg per kg of patient weight administered between about 1 and 10 times per day. In a more preferred embodiment the dosage is between about 1-10 mg per kg of patient weight administered between about 3 and 5 times per day. The frequency of dosing will depend on pharmacokinetic parameters of the IL-1ra in the formulation used.

A preferred dosage range for the treatment of interleukin-1 mediated septic shock is between about 1.0-200 mg per kg per day of patient body weight per 24 hours administered in equal doses between about 4-15 times per 24

hours. In a more preferred embodiment the dosage is between about 10-120 mg per kg per day of patient body weight administered in equal doses every 2 hours. In the most preferred embodiment 100 mg per kg of patient body weight per 24 hours is equally administered every 2 hours. The frequency of dosing will depend on pharmacokinetic parameters of the IL-1ra in the formulation used.

In an additional preferred mode for the treatment of interleukin-1 mediated septic shock, an initial bolus injection of IL-1ra is administered followed by a continuous infusion of IL-1ra until circulating IL-1 levels are no longer elevated. The goal of the treatment is to maintain serum IL-1ra levels between 2-20 ug per ml for this period. In a preferred embodiment of this mode, an initial bolus of between about 10-20 mg per kg of patient body weight of IL-1ra is administered followed by the continuous administration of IL-1ra of between about 5-20 ug per kg of patient body weight per minute until circulating IL-1 levels are no longer elevated. Serum IL-1b levels may be ascertained by commercially available immunoassay test kits. The initiation of treatment for IL-1 mediated septic shock should be begun, under either mode of treatment, as soon as possible after septicemia or the chance of septicemia is diagnosed. For example, treatment may be begun immediately following surgery or an accident or any other event that may carry the risk of initiating septic shock.

A preferred dosage range for the treatment of interleukin-1 mediated ischemia and reperfusion injury is between about 1-50 mg per kg of patient weight administered hourly. In a preferred embodiment an initial bolus of about 15-50 mg per kg of IL-1ra is administered, followed by hourly injections of about 5-20 mg per kg. The frequency of dosing will depend on pharmacokinetic parameters of the IL-1ra in the formulation used.

- It is also contemplated that certain formulations

containing IL-1ra are to be administered orally. Preferably, IL-1ra which is administered in this fashion is encapsulated. The encapsulated IL-1ra may be formulated with or without those carriers customarily used in the compounding of solid dosage forms. Preferably, the capsule is designed so that the active portion of the formulation is released at that point in the gastro-intestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients may be included to facilitate absorption of the IL-1ra. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

When used for the treatment of interleukin-1 mediated IBD, the administration of IL-1ra may also be accomplished in a suitably formulated enema.

Regardless of the manner of administration, the specific dose is calculated according to the approximate body weight or surface area of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein. These dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data.

It should be noted that the IL-1ra formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

It is understood that the application of teachings

of the present invention to a specific problem or environment will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of representative uses of the present invention appear in the following examples.

In Examples 1-3, IL-1ra's are shown to prevent and/or neutralize, either totally or in part, the effects of IL-1 in known arthritis models such as those described by Wilder, R.L., in "Experimental Animal Models of Chronic Arthritis," Goodacre, J.A. and W.C. Dick (ed.), Immunopathogenic Mechanisms of Arthritis, Kluwer Academic Publishers; Dordrecht, Netherlands; Boston, Mass. (1988), specifically incorporated herein by reference. As is noted in the examples, in each model the IL-1ra tested showed beneficial results.

In Examples 4-6, IL-1ra's are shown to prevent and/or neutralize, either totally or in part, the effects of IL-1 in known IBD models such as those described by Zipser et al. Gastroenterology, 92, pp. 33-39 (1987), specifically incorporated herein by this reference; and Sartor et al. Gastroenterology, 89, pp. 587-95 (1985), specifically incorporated herein by this reference. As is noted in the examples, in each model the IL-1ra tested showed beneficial results.

In Example 5, IL-1ra is shown to have beneficial effects on extraintestinal inflammation associated with the Sartor IBD model. In Example 6, intestinal inflammation resulting from the administration of an NSAID--indomethacin--is also neutralized by treatment with IL-1ra.

In Example 7, IL-1ra's are shown to prevent and/or neutralize, in part, the effects of IL-1 in a known septic shock model. Rabbits are given intravenous injections of endotoxins--believed to be a principal cause of septic shock--to induce septic shock. The mortality rate of groups

of rabbits given varying amounts of IL-1ra were examined. As can be seen in the Example, treatment with IL-1ra showed beneficial results.

In Example 8, IL-1ra's are shown to neutralize, in part, the effects of IL-1 in ischemia and reperfusion injury experiments. Dogs are subjected to regional myocardial ischemia for two hours and then reperfused for four hours. The size of infarct as a percentage of ventricular mass and the infarct size as a percent of mass at risk were measured. Treatment with IL-1ra showed beneficial results.

EXAMPLE 1: Demonstration of the Effects of Human Interleukin-1 Inhibitor in Cultured Bovine Nasal Cartilage Explant.

Numerous in vitro and in vivo methods have been used to study the progression of arthritis. One in vitro model which has proven to be especially useful in this regard is cultured cartilaginous tissue explant. In fact, this model has been used in the past to demonstrate that IL-1 is a powerful mediator of cartilage destruction and, therefore, a propitious target for intervention in arthritic joint erosion. (See generally G. Buchan et al., Third Annual General Meeting of the British Society for Rheumatology, London, England, November 19-21, 1988, PR. J. Rheumatol 25 (Supplement 2) 1986; Fontana et al., Rheumatol Int (1982) 2:49-53; J. Saklatvala et al., Development of Diseases of Cartilage and Bone Matrix, Alan R. Liss, Inc., pp. 291-298; P. Stashenko et al., The American Association of Immunologists, Vol. 138, pp. 1464-1468, No. 5, March 1, 1987; G. Dodge et al., J. Clin. Invest., 83:647-661; J. Sandy et al., Journal of Orthopedic Research 4:263-272, J. Saklatavala et al., The Control of Tissue Damage, Glauert, ed., Elsevier Science Publishers, pp. 97-108; I. Cambell et al., Biochem. J. 237:117-122; J. Tyler, Biochem. J., 225:493-507; and J. Eastgate et al., Sixth International

Lymphokine Workshop, 7(3):338, all of which are specifically incorporated herein by reference.)

The cartilage explant model essentially as described by Steinberg *et al.*, Biochem. J. 180:403-412, incorporated herein by reference, was used in this Example to demonstrate the mitigating effect of IL-1ra on IL-1 mediated cartilage breakdown. While bovine nasal septum was used here as the source of cartilaginous tissue, articular cartilage of the type described in J. Tyler *et al.*, Br. J. Rheumatol. 24 (Supplement 1):150-155, incorporated herein by reference, could also be used.

Preparation of Cartilage.

Bovine nasal septum was removed from freshly slaughtered yearling steers and placed on ice. The tissue was then scrubbed with Povidone/Iodine prep solution (1-ethanol-2-pyrrolidinone homopolymer with iodine, obtained from Medline Industries, Mundelein, Ill). The mucosa and perichondrium were then removed. The remaining cartilaginous septum was then immersed in a 5% (v/v) solution of Povidone/Iodine for one hour at room temperature.

The following procedures were then performed aseptically in a laminar flow hood. The septa were repeatedly rinsed with Gey's Balanced Salt Solution (GIBCO Laboratories, Grand Island, NY). The cartilage sheet was then placed on a sterile surface, and uniform 8 mm plugs were removed using a standard cork borer. Approximately 1-2 mm of the top and bottom surfaces were removed using a razor blade. The plugs were then held in the Gey's Balanced Salt Solution. Plugs taken from different steers were kept separately.

Each plug was then sectioned into several 0.8 mm disks. The cutting device used was an aluminum block of the type described by Steinberg *et al.*, Biochem. J. 180:403-412. The disks produced were consistently between 40 and 50 mg

wet weight. The disks were kept in culture in Delbecco's Modified Eagle Medium plus 10% Fetal calf serum, plus Penicillin, Streptomycin, and Neomycin (all reagents from GIBCO), hereinafter referred to simply as "medium." The cultures were maintained in a 37°C incubator with 5% CO₂.

Representative disks from each steer were then tested for their ability to respond to IL-1B as indicated by the release of glycosaminoglycans (GAG) into the culture medium. (glycosaminoglycans are released from a cell once the cell matrix has been degraded.) The presence of GAG was detected using 1, 9-dimethylenethylene blue as described by Ferndale *et al.*, Connective Tissue Research, 9:247-248, incorporated herein by reference. Disks that responded to 5 ng/ml of IL-1B by increasing output of GAG two fold or greater as compared to an unstimulated basal rate were selected for use in the following experiments. These disks are hereinafter referred to as "IL-1B responsive disks."

IL-1 Dose Response.

This preliminary experiment was performed to determine whether a dose response curve exists to increasing amounts of IL-1B.

First, several of the IL-1B responsive disks were sectioned into quarter slices. (The remainder was set aside for later experiments.) Because responses to IL-1 frequently vary from animal to animal, disk to disk, and slice to slice, the steps of this experiment were designed so that each slice served as its own control.

Second, each slice was incubated in one well of a 48 well tissue culture cluster (Costar, Cambridge, MA) with a constant volume of the previously described medium. After 48 hours, the amount of GAG present in the supernatant of each culture was measured. This amount was then normalized for each culture in terms of ug GAG per mg wet weight of tissue. In this manner, a basal rate of GAG release in the absence of IL-1 was established for each slice.

Third, the supernatants from all the cultures were discarded and replaced with fresh medium containing differing amounts of IL-1B. The IL-1B was produced in-house (J. Childs, notebook 935, pages 49-52) and after characterization, was utilized in all experiments calling for its use. After a 48 hour incubation with IL-1B, the supernatants from the cultures were recovered, and the amount of GAG present in each was measured. These amounts were normalized for each culture as above. The basal rates were then subtracted from the IL-1B induced rates. The results are depicted in Fig. 1. (The results are also expressed in tabular form in Table 1.) As Fig. 1 clearly indicates, the release of GAG from the cartilaginous tissue is dependent on the amount of IL-1B administered.

Because 5 ng/ml of IL-1B caused an easily measurable increase in GAG release during the 48 hour period of culturing, this concentration was used in the following experiment.

Effects of rIL-1ra on IL-1 Induced GAG Release.

Several of the remaining IL-1B responsive disks were next sectioned into quarter slices. As above, each slice was used at its own control.

Each slice was then incubated with a constant volume of the previously-described medium for 48 hours in a 48 well tissue culture cluster. A basal rate of GAG release was determined for each slice. Next, the supernatants from the cultures were discarded and replaced with fresh medium containing 5 ng/ml of IL-1B and differing amounts of recombinantly produced IL-1ra (rIL-1ra). After a 48 hour incubation, the supernatants were recovered, and the amounts of GAG were measured. These amounts were normalized for each culture by dividing the rIL-1ra/IL-1B stimulated GAG release rate by the basal GAG release rate. The results are depicted in Fig. 2. As Fig. 2 clearly illustrates, the release of GAG from cartilaginous tissue was sharply

curtailed by an increase in the concentration of rIL-1ra relative to that of IL-1B. For instance, a ten times molar excess of rIL-1ra over IL-1B (the molecular weights of IL-1B and rIL-1ra are both approximately 17 kD) was sufficient to return the GAG release rate to the basal level. Similarly, a 1.5 times molar excess of rIL-1ra over IL-1B was sufficient to reduce the stimulation of GAG release to 50% of that observed in the presence of IL-1B alone.

These results were reproduced using cartilage derived from several different steer.

Lack of Cytotoxicity of rIL-1ra.

To show that rIL-1ra is noncytotoxic, the inventors took slices from the remaining IL-1B responsive disks and exposed them to varying amounts of rIL-1ra in the absence of IL-1B. The rate of GAG release was the same as where neither rIL-1ra nor IL-1B was present.

Next, to show that the effects of IL-1ra are reversible, the inventors then removed rIL-1ra from the supernatants of culturing slices and administered IL-1B thereto. The slices responded just as they did in the IL-1B dose response experiment. Similar results occurred when cartilage that had been treated with IL-1B and a sufficient concentration of rIL-1ra to completely block the action of IL-1B was subsequently exposed to IL-1B alone.

Example 2: Demonstration of the Effects of Human Interleukin-1 Inhibitor on Collagen-Induced Arthritis in Mice.

Type II collagen-induced arthritis in mice bears many resemblances to human rheumatoid arthritis and has been used for several years to study certain aspects of that disease. J. Stuart et al., The FASEB Journal, Vol. 2, No. 14, pp. 2950-2956, November 1988, incorporated herein by reference. The potential involvement of IL-1 in rheumatoid arthritis has been noted by S. Stimpson et al., The Journal

of Immunology, Vol. 140, pp. 2964-2969, No. 9, May 1, 1988, also incorporated herein by reference.

The purpose of this experiment was to demonstrate that systemic administration of rIL-1ra has a mitigating effect on the pathogenesis of type II collagen-induced arthritis in mice.

Twenty-four mice DBA/1 mice, purchased from Jackson Laboratories, were immunized with 0.1 mg chick type II collagen in Freund's complete adjuvant. At day fourteen post immunization, the animals were randomly subdivided into two groups of twelve animals each. The experimental group was injected intraperitoneally twice daily with approximately 0.1 mg rIL-1ra/kg/injection. The injections continued until the animals were sacrificed at day 47 post immunization (i.e., after 34 days of dosing). Control animals were injected with an equal volume of vehicle (10 mM sodium phosphate, 150 mM sodium chloride) on the same schedule.

Affected limbs were counted and clinical scoring was performed approximately three times weekly during the in-life portion of the experiment. Clinical scores from each animal represent, on a 0-4 point basis, the severity of arthritis sustained by each paw as assessed by blinded observers. The clinical scores for each animal from day 26, when the first signs of clinically observable arthritis were noted, through day 47, when the animals were sacrificed, are presented in Table 2. The tally of affected limbs and the total clinical score for each group are also presented in Table 2. These results are graphed as a function of time in Figs. 3 and 4, respectively. As can clearly be seen, the incidence and severity of the disease were slowed down considerably by the administration of rIL-1ra.

Example 3: Demonstration of the Effects of Human Interleukin-1 Inhibitor on Streptococcal Cell Wall (SCW)-Induced Reactivation of SCW-Induced Arthritis in Rats

Regarding streptococcal cell wall-induced arthritis, R. L. Wilder in Immunopathogenetic Mechanisms of Arthritis, Chapter 9 entitled "Experimental Animal Models of Chronic Arthritis" comments "the clinical, histological and radiological features of the experimental joint disease closely resemble those observed in adult and juvenile rheumatoid arthritis".

The experiment described below employs the model disclosed in Esser, et al. Arthritis and Rheumatism, 28: 1401-1411, 1985, specifically incorporated herein by reference. This model is briefly summarized as follows: Streptococcal Cell Wall (SCW) is injected intraarticularly into the ankle joint of Lewis rats. Saline is injected into the contralateral joint to provide a control. After a period of twenty days, in which the initial inflammation dies away, SCW is again administered, this time by intravenous injection. This dose of SCW is insufficient to cause joint inflammation by itself and, therefore, has little or no effect on the saline injected ankle. In contrast, however, this dose is capable of reactivating inflammation and joint destruction in the ankle previously injected with SCW. To assess the extent of inflammation following the second administration of SCW, the dimensions of the ankle joint are measured daily.

In one of many experiments performed with the above described model, two groups of twelve rats were used. Each animal was injected in the right ankle with SCW (1.8 ug rhamnose equivalence) and in the left ankle with an equal volume of pyrogen-free saline. Ankle dimensions were measured on days 1 through 6.

On day 20, one group of rats was injected

intraperitoneally with 1 mg/kg IL-1ra in an aqueous vehicle; the other group was injected intraperitoneally with an equal volume of the vehicle solution only. One hour later, each animal was injected intravenously with SCW (100 ug rhamnose equivalence). Ten minutes later, the treatment group was injected intraperitoneally with 1 mg/kg IL-1ra, and the control group was injected with vehicle alone. Subcutaneous injections of IL-1ra at 1 mg/kg were given at 2 and 6 hours post-SCW administration and were repeated every 6 hours thereafter for the next 3 days.

Table 3 and Figure 5 show the dimensions of the saline injected and SCW injected ankles for both the treatment group and the control group over the course of the experiment. As expected, the SCW treated ankles in both groups swelled in response to the intravenous injection of SCW. However, the response differed between treatment groups. The ankles in the control group swelled by about 30% of their initial dimensions over the first 3 days whereas the ankles in the treatment group swelled only by 14% over the same period. Moreover, on days 1 through 5 post-intravenous injection of SCW, there was a statistically significant ($P < 0.001$ by a two-tailed t-test for independent means) difference in the dimensions of the SCW-treated and contralateral control ankles both groups.

On day 8, the rats were sacrificed and both ankles were fixed in formalin. The fixed joints were decalcified, stained, and examined. Significant differences in cartilage erosion, bursitis, periostitis, and synovitis were found between the control group and the treatment group. Some of these differences are set forth in Table 4.

Example 4: Demonstration of the Effects of Human Interleukin-1 Inhibitor on Formalin-Immune Complex Induced IBD.

The rabbit model of formalin-immune complex IBD

has been used to investigate the role of arachidonic acid-derived inflammatory mediators and to evaluate therapeutic strategies in IBD. Zipser et al. supra; Brown et al. 1987 Gastroenterology 92:54-59; Schumert et al. 1988 Prostaglandins 36:565-577, incorporated herein by reference.

The experiment described below employs the model disclosed in Zipser et al. supra. This model creates symptoms analogous to active Ulcerative Colitis, and is briefly summarized as follows: formaldehyde is administered via a catheter into the colon of rabbits and after a period of time the animals receive an injection of immune complexes in antigen excess. Time studies following the induction of IBD are conducted by sacrificing the animals after 48 hours and removing the colons. The colons are then histologically assessed. The effect of treatment with IL-1ra's prior to and after the induction of IBD on inflammation, edema and necrosis was compared with non-treated control animals.

Induction of IBD.

Inflammation was induced in the distal colon of male New Zealand rabbits (2.2-2.5kg) using a modification of the immune complex method of colitis Kirsnew et al. 1957 Trans. Assoc. Am. Physicians 70:102-119; Hodgson et al. 1978 Gut 19:225-32, which are incorporated herein by reference. Four ml of 0.45% (v/v) unbuffered formaldehyde (Electron Microscopy Sciences, Washington, PA) was administered via a catheter inserted 10 cm into the distal colon of anesthetized rabbits (xylazine and ketamine). Two hours later, animals received 0.85 ml of immune complexes in antigen excess through an ear vein. The complexes were prepared by incubating human serum albumin (500ug/ml) with rabbit antihuman antisera (ICN Immunobiologicals, Costa Mesa, CA), decanting the supernatant, and redissolving the precipitated immune complexes with an albumin solution (6mg/ml) as described in Zipser et al. supra.

Histologic evaluation was performed on a minimum

of two longitudinal sections from each colon. All colon samples were examined in a blind fashion by a single pathologist. The mucosa and submucosa were separately evaluated for infiltration of acute inflammatory cells (neutrophils and eosinophils). A semiquantitative score of leukocytes (L) per high power field (HPF) was determined for each area examined using the following quantitations: 0 = 0 or 1; 0.5 = 2-9; 1 = 10-20; 1.5 = 21-30; 2 = 31-40; 2.5 = 41-50; 3 = 51-65; 3.5 = 66-80; 4 = > 81 L/HPF. At a minimum, eight HPFs of mucosa and submucosa from each specimen were separately evaluated in each section. The inflammatory index was calculated by adding the averaged score for the mucosal and submucosal evaluations. Edema was semiquantitatively assessed on a scale of 0 to 4. Necrosis was expressed as the percent of mucosa involved. After the administration of formalin, followed by immune complexes, the distal colon develops acute inflammation. This is characterized by infiltration of neutrophils primarily into the mucosa and submucosa, mucus depletion, crypt abscesses, edema and scattered areas of mucosal necrosis, progressively increased from 0.3 ± 0.1 (0 hrs) to 4.5 ± 0.7 (48 hrs) ($p < 0.001$), from 0.3 ± 0.1 to 3.6 ± 0.3 ($p < 0.001$) and from 0% to 89% ($p < 0.001$) respectively. A subsequent decrease in these parameters was observed 96 hours after the induction of IBD ($p < 0.01$ versus 48 hours).

Treatment with IL-1ra's.

A group of animals were treated intravenously with IL-1ra (5mg/kg; n=8) or the vehicle alone (n=10) at six time points: 2 hours before and 1, 9, 17, 25, 33 hours after the administration of the immune complexes. The rabbits were sacrificed 48 hours after the induction of IBD and the colon tissue analyzed for inflammation.

Treatment of rabbits with IL-1ra significantly reduced inflammatory index from 3.2 ± 0.4 to 1.4 ± 0.3 ($p < 0.02$),

edema from 2.2 ± 0.4 to 0.6 ± 0.3 ($p < 0.01$) and necrosis from $43 \pm 10\%$ to $6.6 \pm 3.2\%$ ($p < 0.03$) compared to vehicle-treated IBD animals, FIG. 6. This result shows that several of the indications of IBD may be significantly lessened by treatment with IL-1ra.

Example 5: Bacterial Cell Wall Induced IBD in Rats

Unlike many other IBD models, the bacterial cell wall induced IBD model shows most of the indications for chronic IBD or Crohn's disease. In addition to the formation of chronic granulomatous response, this model is subject to spontaneous reactivation, anemia and extraintestinal inflammation.

The Bacterial Cell Wall model essentially as described by Sartor et al. supra., was used in this Example to demonstrate the mitigating affect of IL-1ra on IL-1 mediated IBD. The experiment was performed generally as follows: the IBD is induced in rats by the intravenous injection of a sterile sonicate of peptidoglycan polysaccharide from group A streptococci. Transient petechial hemorrhage of the colon appears within 2-3 minutes and resolves by 48-72 hours after injection. A sample group of animals were treated with IL-1ra following induction of IBD, and after a period of time the animals were sacrificed, the colons removed and gross pathology evaluated.

Induction of IBD.

The bacterial cell wall material was prepared according to the procedures set forth in Stimpson et al. 1986 Infect. Immun. 51:240-249, incorporated herein by this reference. Lewis rats are given subsercosal injections with Streptococcal Cell Walls. The injections result in both local and systemic disorders that include bowel adhesions and nodules, an increased liver weight and hepatic nodules, a reduced hematocrit and hemoglobin level, and increased white blood cell count (WBC), a reduced growth rate, and a

joint swelling characteristic of arthritis (see Appendix Sartor, et al., Gastroenterology, 89:587-595, 1985, incorporated herein by reference). Three separate protocols for treatment with IL-1ra were performed with this model and reductions in nodules and adhesions have been observed in all of them; in the last two protocols the reductions in adhesions were statistically significant.

Protocol A.

Two groups of 12 rats were used. On day 1, both were injected with 15 ug total of Streptococcal cell wall derived peptidoglycan polysaccharide (SCW PG-APS), at seven sites; three areas of the cecum, 2 areas of the Peyer's patches, and two areas of the ductal ileum. On day 11 overt signs of the disease appeared including joint swelling, diarrhea, and bloody nose. At this time one group was dosed subcutaneously with IL-1ra (8 mg/kg) every 12 hours and the second group was treated identically with placebo (PBS). On each day the size of the ankle joints were measured. On day 18 the animals were sacrificed and the intestines were scored on a scale of 0 to 4 for the presence of granulomas and adhesions (Table 5). The IL-1ra group had fewer nodules and adhesions. The IL-1ra group also had smaller livers. The IL-1ra group had a reduced white blood cell count (WBC).

Protocol B.

The protocol was similar to that used in protocol A except that the amount of PG-APS used was reduced to 12.5 ug and the treatment with IL-1ra was started at day 8. As in protocol A, reductions in cecal nodules, intestinal adhesions, liver weights, and WBC were observed (Table 6). The reduction in adhesion was significant at the $p < 0.02$ level.

Protocol C.

The protocol used was again similar to that in protocol A except that the amount of PG-APS was reduced to 12.5 ug (as in protocol B) and the treatment group was

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started on IL-1ra 8 mg/kg subcutaneous and 2 mg/kg i.v. immediately following the PG-APS injection. Further IL-1ra injections (8 mg/kg) s.c. were given at 4, 10 and 18 hours on day 1, every 8 hours on day 2, and then every 12 hours for the duration of the experiment. Five animals in each group were sacrificed at day 3, and the remainder were sacrificed at day 18 for examination of gut lesions (Table 7). On day 3 there was a significant reduction in a global parameter representing gut lesions and a reduction in adhesions that approached significance ($p = 0.07$). In the group sacrificed at day 18 the results were confused because no disease appeared in one of the animals in the control group. However, the reduction in adhesions in the IL-1ra group was still significant at the $p < 0.02$ level and there was also a significantly greater weight gain in the IL-1ra group.

EXAMPLE 6: NSAID Induced IBD in Rats.

In an attempt to determine whether the anti-inflammation effects of IL-1ra would be additive with those of NSAIDs, rats were treated with indomethacin after the intravenous injection of PG-APS as described in Example 3 above (2 mg/kg/ at the time of reactivation at 12, 24 and 36 hours post activation, and every 12 hours up to 6 days), IL-1ra (2 mg/kg at 2 and 6 hours, then every 6 hours up to 36 hours and every 12 hours up to 7 days) or a combination of the two drugs (Figure 7). The group on indomethacin alone showed a greater reduction in joint swelling than that on IL-1ra alone. However, the indomethacin group was sick and two animals died during the course of the experiment. The group receiving both drugs did even better than the group on indomethacin alone; the joint swelling was less, and the difference between the two groups was statistically significant on day 4 at $p < 0.03$ and on day 7 and 8 at $p < 0.06$. No animals were sick in this group and there were -

fewer ulcerations in the mid small intestines. Ulceration of the mid small intestine is a complication in patients on chronic oral NSAIDs. It appears, therefore, that IL-1ra alleviates some of the IBD-like complications of NSAIDs.

Table 8 shows the effects of IL-1ra on both the intestinal symptoms--ulcers, adhesions, intestinal thickening and myeloperoxidase (MPO) levels--and systemic symptoms--hematocrit (HCT), hemoglobin (Hgb) and WBC levels--associated with the NSAID treatment of PG-APS induced arthritis.

Example 7: Demonstration of the Effects of Human Interleukin-1 Receptor Antagonist on Endotoxin Induced Septic Shock.

Endotoxin induced septic shock studies were conducted on Blue Chinchilla rabbits. The experimental protocol did not focus on any indications of the induced septic shock other than group mortality. Rabbits were used in the study because their sensitivity to pyrogenic and metabolic effects of endotoxin and other bacterial products are similar to those of human subjects.

Shock was induced by a single intravenous injection of endotoxin at time zero. The rabbits were given periodic intravenous injections into an ear vein at -10 min., at time zero, and for every two hours thereafter for a 24 hour period. The results of this study can be seen in Table 9.

In Table 9, Group A rabbits (n=5) were not given any endotoxin at time zero, and were given saline injections free of IL-1ra at the periodic injection times. Group B rabbits (n=10) were given .5 mg per kg of body weight of endotoxin at time zero, and the periodic injections were again free of IL-1ra. After 7 days the survival rate of rabbits in Group B was only 20%.

In Groups C-E (n=10) endotoxin was administered at time zero, and the saline injections contained varying amounts of IL-1ra. The rabbits in group C received a total of 10 mg per kg of body weight of IL-1ra. The rabbits in group D received a total of 30 mg per kg of body weight of IL-1ra. And finally, the rabbits in group E received a total of 100 mg per kg of body weight of IL-1ra. After 7 days, the survival rate of rabbits in group E was 90%.

This experiment, graphically illustrated in Figure 8, shows that treatment with IL-1ra significantly delays and reduces final mortality rates in rabbits with endotoxin induced shock.

Example 8: Demonstration of the Effects of Human Interleukin-1 Receptor Antagonist on Ischemia and Reperfusion Injury

In the following example experimental dogs were subjected to regional myocardial ischemia for two hours and then reperfused for four hours. The dogs were divided into two groups, one group treated with IL-1ra and the other treated with serum albumin in the same buffer used for the test group.

Animals were fasted overnight and on the following morning, were anesthetized with 10 ml of thiamylal sodium 5%, followed by 2 ml of sodium pentobarbital 6%, intravenously. Additional sodium pentobarbital was administered during the experiment as necessary. Artificial respiration was maintained with a Harvard respirator. A left thoracotomy was performed through the fifth intercostal space and polyvinyl catheters placed in the left internal jugular vein for fluid and drug administration, and in the left internal carotid artery and femoral arteries for pressure monitoring and withdrawal of reference blood samples. A catheter was placed in the left atrium for injection of radio active microspheres. The left circumflex

artery was dissected free of surrounding tissue and an electromagnetic flow probe was placed on the vessel proximal to the first obtuse marginal branch. After an intravenous bolus injection of 50 mg of lidocaine, the circumflex coronary artery was occluded with the snare occluder for 2 hours. Complete occlusion was verified with the electromagnetic flow probe. The snare was then released suddenly, allowing reperfusion of the coronary vascular bed for 4 hours.

Two-dimensional echocardiograms and hemodynamic measurements (heart rate, blood pressure and left atrial pressure) was determined before occlusion, after 110 min of occlusion, 5 minutes after reperfusion, and 4 hours after reperfusion. Two-dimensional echocardiography was performed with the use of a scanner and a 2.25 MHz transducer. The transducer was placed on the closed shaved right chest and was allowed full visualization of the circumferential extent of the left ventricle in a short-axis projection. Echocardiographic images were recorded at the midpapillary muscle position onto a video cassette with use of a Sony recorder. A two-dimensional echocardiographic analysis was performed with the use of a minicomputer-based video digitizing system.

End-diastolic and end-systolic frames were selected for analysis with the use of the onset of the Q wave in lead II as a marker of end-diastole and the smallest left ventricular cavity size as a marker of end-systole. Endocardial and epicardial borders for 3 consecutive beats during normal sinus rhythm was carefully traced directly from the video display onto a digitizing tablet. Quantitative analysis was performed with a radial contraction model and a fixed diastolic center of mass at 22.5 degree intervals over the full left ventricular circumference.

The midpoint of the posterior papillary muscle was chosen as a fixed anatomic reference and designated as 135°

degrees. Wall thickening was computed for each of the 22.5 degree sectors with the following equation: wall thickening = [(end systolic wall thickness - end diastolic wall thickness) / end diastolic wall thickness] x 100%. The normal range of wall thickening was determined from a functional map of the baseline images for three cardiac cycles and 95% tolerance limits were established in each animal. These limits were used for comparison with occlusion and reperfusion functional maps and abnormalities are expressed as the circumferential extent of dysfunction and the degree of dysfunction. The extent of dysfunction (in degrees) was measured at the intercepts between the occlusion or reperfusion maps and the lower 95% tolerance limit; the degree of dysfunction (in area units) is the planimetered area below the lower 95% tolerance limit.

Regional myocardial blood flow was assessed by the reference withdrawal method using tracer-tableted microspheres (15 μ m diameter, New England Nuclear) injected into the left atrium. The microspheres were ultrasonicated and vortex-agitated before injection. Microspheres were injected before occlusion, after 110 min of occlusion, 5 minutes after reperfusion and 4 hours after reperfusion with one of six available isotopes (^{141}Ce , ^{51}Cr , ^{113}Sn , ^{103}Ru , ^{95}Nb , ^{46}Sc). Simultaneous reference arterial samples were withdrawn from the carotid and femoral arteries at a constant rate of 7 ml/min with a Harvard withdrawal pump starting 10 sec before microsphere injection and continuing for 120 sec after completion of the injection.

Two adjacent transverse left ventricular slices at the midpapillary muscle level, corresponding to the echocardiographic short-axis slices, were selected for blood flow determination. Each slice was divided into 16 full-thickness 22.5 degree sectors. Each sector was then further divided into epicardial, midmyocardial, and endocardial samples. The tissue samples were then weighed, placed in

counting vials, and assayed for radioactivity in a gamma scintillation counter. After background and overlap correction, absolute myocardial blood flow was calculated with the following equation: $Q_m = (C_m \times Q_r / C_r)$, where Q_m = myocardial blood flow (ml/min); C_m = counts/min in tissue sample; Q_r = withdrawal rate of the reference arterial sample (ml/min); C_r = counts/min in the reference arterial sample. Myocardial blood flow is expressed per gram of tissue for each sample.

Just prior to sacrifice, the left circumflex coronary artery was briefly occluded and monastral blue pigment (0.5 ml/kg) injected into the left atrium for delineation of the in vivo myocardial area at risk. The animal then received 3000 U of heparin and was sacrificed with an intravenous bolus of saturated KCl solution and the heart excised.

Treatment Groups Dogs were randomly assigned to one of two groups. In the first group, dogs received a bolus injection of 30 mg IL-1ra inhibitor just prior to the onset of the ischemia and 15 mg IL-1ra inhibitor for each hour until the experiment was terminated. Control animals received an identical quantity of endotoxin-free, human albumin dissolved in the same buffer used for the test group.

Determination of Infarct Size. After death, the heart of each dog was excised, the left ventricle isolated from surrounding tissue, cooled in a freezer for 15 minutes, and then sliced into 5 mm transverse sections. The slices were then weighed and placed in a warm bath of buffered triphenyl tetrazolium chloride for ten minutes. In this technique, viable tissue stains red while nonviable tissue remains unstained (Am. Heart. J. 101:593). The unstained zone of infarcted tissue is outlined on transparent overlays and quantitated by planimetry using a microcomputer and corrected for the weight of the heart slice. Infarct size,

is expressed as the percentage of the area of myocardium at risk (the area at risk of infarction is defined as the area of the myocardium left unstained following the injection of monastral blue into the left atrium).

NMR Analysis of myocardial Edema. After fixation, the hearts were cut into 5 to 7 transverse slices approximately 5 mm thick. Two transmural myocardial tissue samples were obtained from the nonischemic zone (positive monastral blue staining) and the central ischemic zone (negative blue staining). The epicardium for each sample was dissected away to eliminate possible lipid signal interference. Each piece was subdivided transmurally (weighing approximately 500 mg each) with one portion assessed for % H₂O by desiccation technique (wet weight - dry weight/wet weight) while the other was placed into a clean dry glass tube. T1 and T1 relaxation times were obtained on a IBM PC 20 Minispec spectrometer (IBM Instruments, Inc., Danbury, CT) operating at 20 MHz and 40°C. The location of the sample in the magnet, 90° and 180° radio frequency pulses, and detector phase were optimized for each sample before relaxation measurements were obtained. T1 values were determined by a fit of 20 inversion data recovery points while T2 values were determined by using a Carr, Purcell, Meiboom-Gill (CPMG) sequence. In an attempt to minimize effects of diffusion and miscellaneous system instabilities, the 180° radio frequency interpulse spacing was maintained at 180 microseconds. The fraction of echo samples determined were used as variables to adjust the duration of the CPMG experiment. Typically, 1 to 150 data points were acquired as the echo train was delayed to 15% to 25% of its original amplitude. T2 values were determined by using a multi-exponential fit. Only the dominant component of the exponential fit was used for statistical analysis. The results of the T1 and T2 analysis were corrected with

percent water for adjacent tissue samples to verify the accuracy of the NMR technique.

Histologic and morphometric Evaluations. For each group tested at least 3 animals were evaluated by light microscopy. Sections stained with hematoxylin and eosin from each heart were evaluated for neutrophil accumulation within the area between viable and infarcted tissue.

Statistic Analysis. All data was represented as the mean \pm SEM. Comparisons within groups were made by a two-way analysis of variance; when significant F values are obtained, paired t tests (corrected for multiple comparisons with the Bonferroni inequality adjustment) will be used to determine which measurements differed significantly from one another.

Comparisons between groups were made by unpaired t test. An exponential regression was used to correlate infarct size data to myocardial blood flow.

The results of the IL-1ra treatment regimen on protecting dog myocardium from occlusion reperfusion injury, are listed in Table 10 below. As a percentage of the left ventricular mass the infarct size in the treated group was reduced to 10.3% as opposed to 18.2% in the control animals. This represents a 40% reduction in the percent of the left ventricular mass that was infarcted. The percentage of the area at risk in contrast, was not markedly changed, 40.5% of the left ventricular mass in the treated group versus 44.8% in the control animals. When the infarcted area is calculated as a percent of the total area at risk, the numbers similarly favor the IL-1ra treated animals, 24.9% versus 42% in the control group.

TABLE 10

The effect of IL-1ra in reducing the extent at infarcted tissue in canine coronary occlusion-reperfusion studies.

<u>IL1ra Treated (n=9)</u>	<u>Albumin Treated (n=9)</u>
Infarct size as a % of left ventricular mass	
10.3% \pm 2.2%	18.2% \pm 3.3%
Area at risk as a % of left ventricular mass	
40.5% \pm 1.7%	44.8% \pm 1.9%
Infarct size as a % of mass at risk	
24.9% \pm 4.6%	42% \pm 8.3%

Although the present invention has been described in connection with preferred embodiments, it is understood that those skilled in the art are capable of making modifications and variations without departing from the scope or spirit of the present invention. Therefore, the foregoing description of preferred embodiments is not to be taken in a limiting sense, and the present invention is best defined by the following claims and their equivalents.

Table 1

The effect of recombinant IL-1 α on IL-1 β induced degradation of Bovine Nasal Cartilage.
N = 6.
In all cases [IL-1 β] = 5 ng/ml.

<u>[IL-1α]</u>	<u>$\frac{[IL-1\alpha]}{[IL-1\beta]}$</u>	<u>Fold Stimulation period II/period I (+/- standard deviation)</u>	
0		4.02	+/- 1.7
5 ng/ml	1	2.4	+/- 0.47
10 ng/ml	2	1.7	+/- 0.4
25 ng/ml	5	1.3	+/- 0.4
50 ng/ml	10	1.0	+/- 0.2
150 ng/ml	30	1.1	+/- 0.2

Individual Clinical Scores on Days
26 through 47 Post Immunization

[illegible]

Table 3

ANKLE JOINT DIAMETER OF RATS INJECTED
WITH SCW AND TREATED WITH IL-1 α OR SALINE
ACCORDING TO PROTOCOL IN EXAMPLE 3

Day	Joint Diameter (mm) (\pm SD)							
	SCW Injected Joints				Saline Injected Joints			
	IL-1 α	SD	Saline	SD	IL-1 α	SD	Saline	SD
0	5.96	.12	6.02	.10	5.95	.17	5.96	.16
1	7.95	.33	7.73	.36	5.94	.15	5.94	.13
2	7.44	.28	7.42	.27	5.98	.11	5.95	.17
3	7.20	.39	7.23	.27	6.00	.12	6.01	.07
6	6.78	.27	6.61	.29	6.06	.09	6.06	.13
10	6.58	.34	6.63	.18	6.00	.12	5.85	.16
14	6.44	.21	6.36	.17	5.99	.08	5.90	.17
20	6.46	.19	6.52	.14	5.91	.11	5.87	.20
21	7.34	.36	7.78	.31	5.73	.18	5.78	.12
22	8.31	.58	8.70	.43	5.85	.16	5.96	.22
23	8.55	.81	9.06	.42	6.02	.19	5.99	.16
24	8.23	.71	8.56	.39	6.03	.13	5.94	.20
25	8.00	.56	8.16	.43	6.05	.12	6.06	.17
28	7.48	.40	7.71	.30	6.04	.13	5.98	.13

Table 4

Effects of IL-1 α on Joint Histopathology
Following SCW Reactivation of Joint Inflammation

(1 mg/kg 4 times daily on day 20 through 23)

Pathology	Placebo Group		IL-1 α Group		P
	Positives/12	Score	Positives/12	Score	
Cartilage Erosion	10	1.0 \pm .6	3	0.25 \pm .45	.0023
Bone Erosion	3	0.25 \pm .45	2	0.17 \pm .39	NS
Bursitis	11	0.92 \pm .29	3	0.25 \pm .45	.0003
Periostitis	9	0.75 \pm .45	12	0.25 \pm .45	.013
Synovitis	12	2.21 \pm .84	12	1.08 \pm .47	.00052
PMN	12	1.0	12	1.0	NS

Table 5

Effects of IL-1ra on SCW-induced enterocolitis
in the rat

	<u>Intestinal Adhesions</u>	<u>Cecal Nodules</u>	<u>Liver Weight (gm)</u>	<u>WBC</u>
IL-1ra	1.7	1.8	16.9	48.8
PBS	2.2	2.4	18.6	57.7
p value for comparison of groups	0.14	0.10	0.19	0.13

Table 6

Effects of IL-1ra on SCW-induced enterocolitis
in the rat

	<u>Intestinal Adhesions</u>	<u>Cecal Nodules</u>	<u>Liver Weight (gm)</u>	<u>WBC</u>
IL-1ra	1.4	1.7	13.3	35.1
PBS	2.2	2.3	14.1	35.8
p value for comparison of groups	0.017	0.077	0.23	0.43

Table 7

Effects of IL-1ra on SCW-induced enterocolitis
in the rat

	<u>Intestinal Adhesions</u>	<u>Cecal Modules</u>	<u>Liver Weight (gm)</u>	<u>WBC</u>
IL-1ra	0.8	0.9	0.047	10.7
PBS	1.8	1.0	0.049	10.3
p value for comparison of groups	0.07	0.30	0.24	0.31

Effects of IL-1ra Treatment on Indomethacin-Induced Gut Injury in the Rat

Treatment	Intestinal Symptoms					Systemic Symptoms		
	Deaths	Ulcers		Adhesions	Intestinal Thickening	MPO u/g	HCT %	Hgb (g/dl)
		#	% Area					
Saline	0/9	0	0	0/9	0/9	0.005 (.001)	42	15
IL-1ra	0/10	0	0	0/10	0/10	0.01 (.01)	41	14
Indomethacin	2/9	1.3 (\pm 0.6)	7.1 (\pm 2.7)	2/7	4/7	0.06 (.02)	37	13.5
Indomethacin + IL-1ra	0/8	0.6 (\pm 0.4)	2.8 (\pm 1.9)	3/8	2/8	0.03 (.02)	40	14
								11

Table 9

Experimental endotoxin induced shock in rabbits; effect of Ultra on survival rate

	survival (no)					survival rate 7 days (%)
	12 h	24 h	36 h	48 h	7 d	
A (N=5)	5	5	5	5	5	100
B (N=10)	9	6	3	2	2	20
C (N=10)	9	7	4	3	2	20
D (N=10)	10	7	6	5	4	40
E (N=10)	10	10	10	9	9	90

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for treating interleukin-1 mediated disease which comprises administering to a patient in need thereof a therapeutically effective amount of an interleukin-1 inhibitor.
2. The method of claim 1 wherein said interleukin-1 inhibitor is a protein.
3. The method of claim 1 wherein said interleukin-1 inhibitor is IL-1ra
4. The method of claim 3 wherein said IL-1ra comprises at least one compound from the group consisting of IL-1raa, IL-1raB, IL-1raX and methionyl IL-1ra.
5. The method of claim 4 wherein said IL-1ra is IL-1raa.
6. The method of claim 4 wherein said IL-1ra is IL-1raB.
7. The method of claim 4 wherein said IL-1ra is IL-1raX.
8. The method of claim 4 wherein said IL-1ra is methionyl IL-1ra.
9. The method of claim 4 wherein said IL-1ra is produced by recombinant DNA methods.
10. The method of claim 9 wherein said IL-1ra is produced in substantially pure form.
11. The method of claim 1 wherein said IL-1 inhibitor is administered in a pharmaceutically acceptable carrier.
12. The method of claim 1 wherein said IL-1 inhibitor is administered in a liquid form.
13. The method of claim 1 wherein said interleukin-1 inhibitor is an IL-1 binding protein.
14. The method of claim 13 wherein said IL-1 binding protein is a soluble receptor.
15. The method of claim 13 wherein said IL-1 binding protein is a monoclonal antibody.

16. The method of claim 1 wherein said interleukin-1 inhibitor blocks IL-1 production.

17. The method of claim 16 wherein said interleukin-1 inhibitor that blocks IL-1 production is IL-1ra.

18. The method of claim 1 wherein said interleukin-1 mediated disease is selected from the group consisting of: arthritis, inflammatory bowel disease, septic shock, ischemia injury, reperfusion injury, osteoporosis, asthma, insulin diabetes, myelogenous and other leukemias, psoriasis, and cachexia/anorexia.

19. A method for preventing interleukin-1 mediated disease comprising administering to a patient in need thereof a therapeutically effective amount of an interleukin-1 inhibitor.

20. The method of claim 19 wherein said interleukin-1 inhibitor is IL-1ra.

21. The method of claim 20 wherein said IL-1ra is selected from the group consisting of IL-1raa, IL-1raB, and IL-1raX.

22. The method of claim 19 wherein said interleukin-1 inhibitor is an IL-1 binding protein.

23. The method of claim 22 wherein said IL-1 binding protein is a soluble receptor.

24. The method of claim 22 wherein said IL-1 binding protein is a monoclonal antibody.

25. The method of claim 19 wherein said interleukin-1 inhibitor blocks IL-1 production.

26. The method of claim 25 wherein said interleukin-1 inhibitor that blocks IL-1 production is IL-1ra.



27. The method of Claim 19 wherein said interleukin-1 mediated disease is selected from the group consisting of: arthritis, inflammatory bowel disease, septic shock, ischemia injury, reperfusion injury, osteoporosis, asthma, insulin diabetes, myelogenous and other leukemias, psoriasis, and cachexia/anorexia.

DATED this 23rd day of June 1993

SYNERGEN, INC.

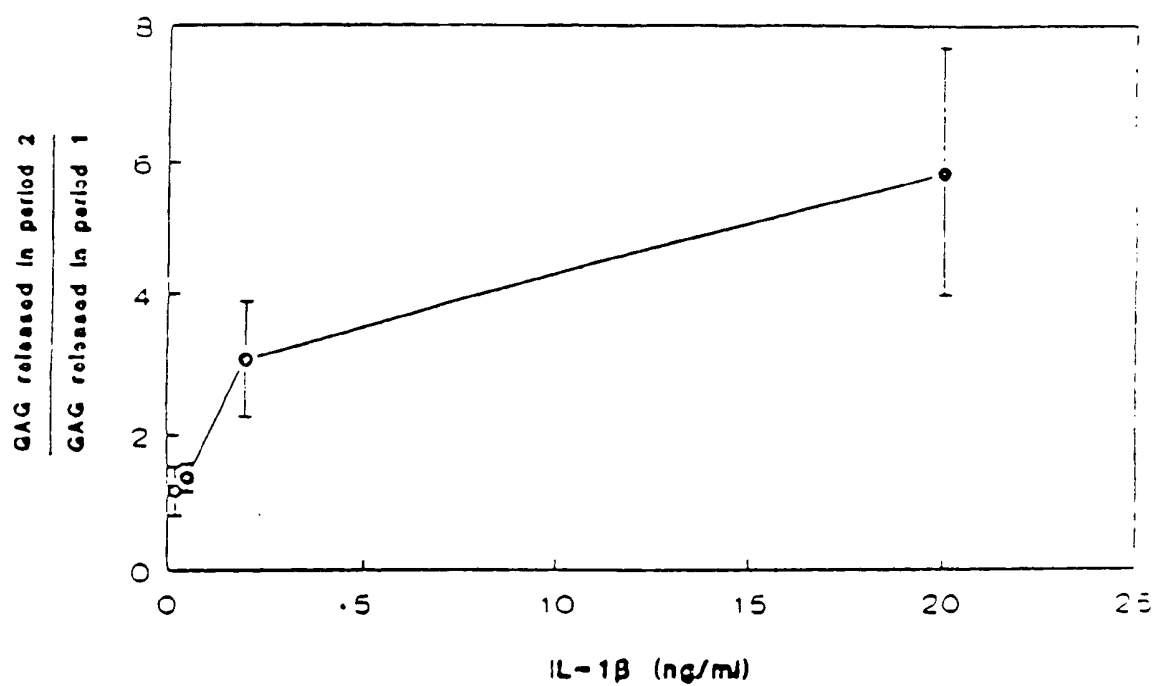
By Their Patent Attorneys:

GRIFFITH HACK & CO

Fellows Institute of Patent
Attorneys of Australia

Figure 1

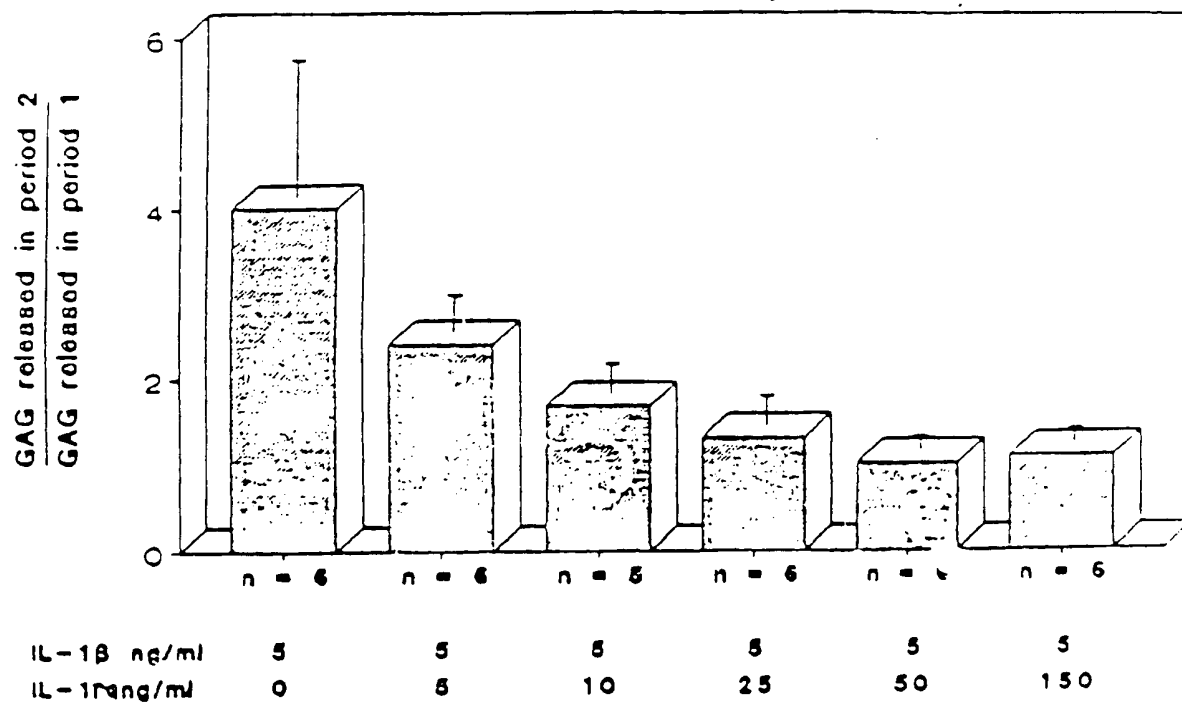
IL-1 β Dose Response Curve



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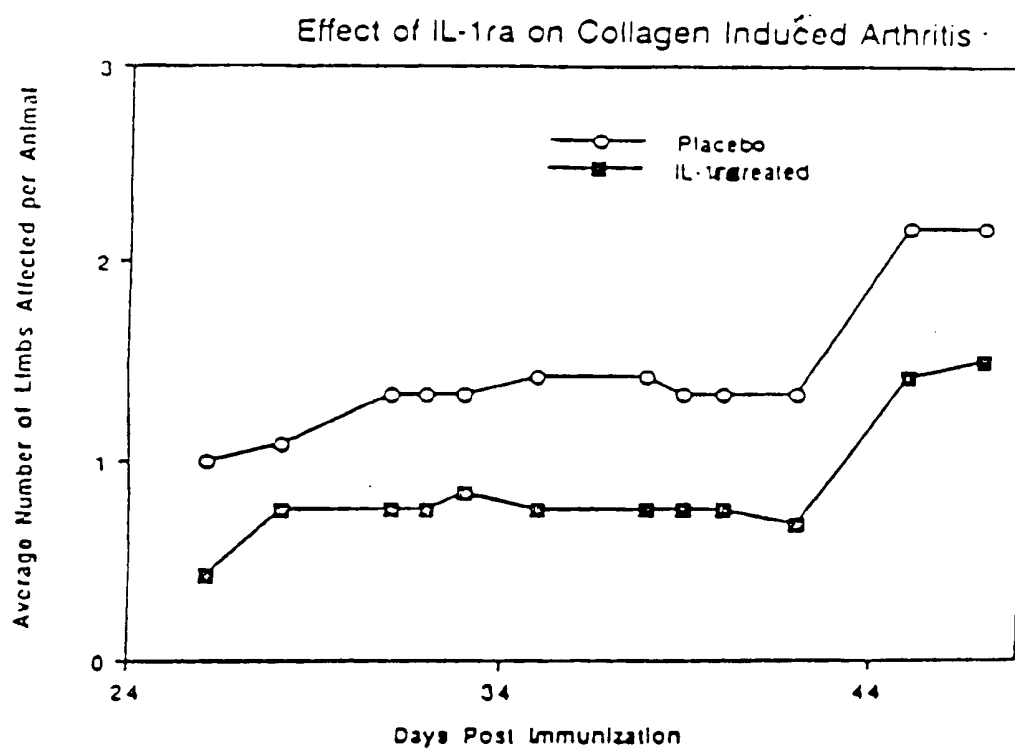
Figure 2

The effect of recombinant IL-1ra on IL-1 β induced degradation of Bovine Nasal Cartilage



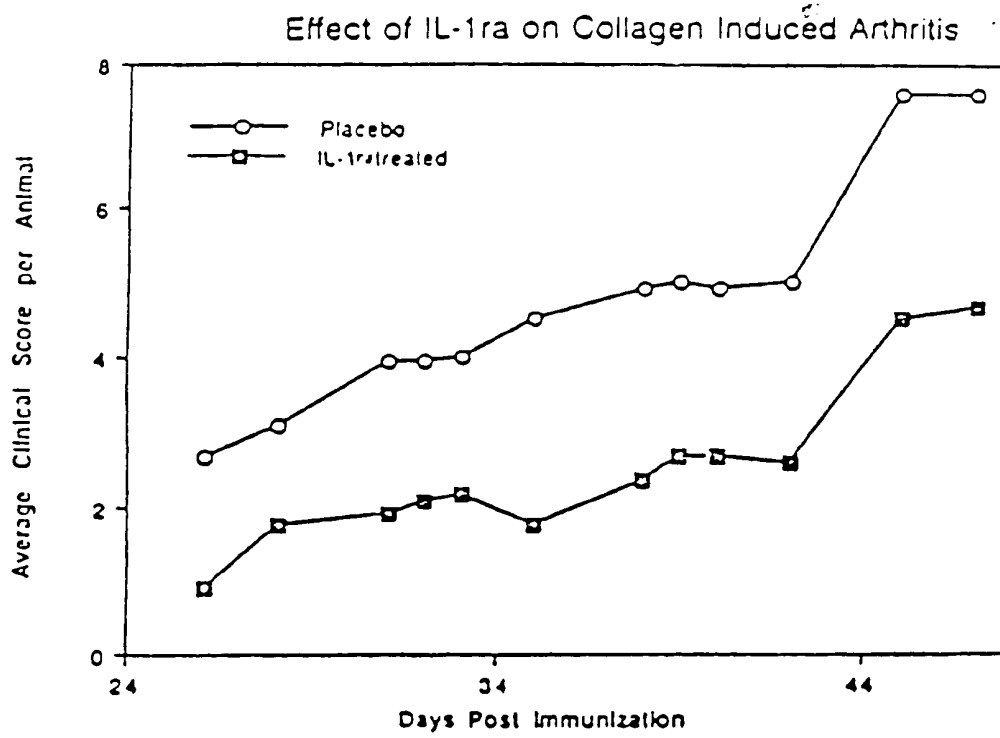
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Figure 3



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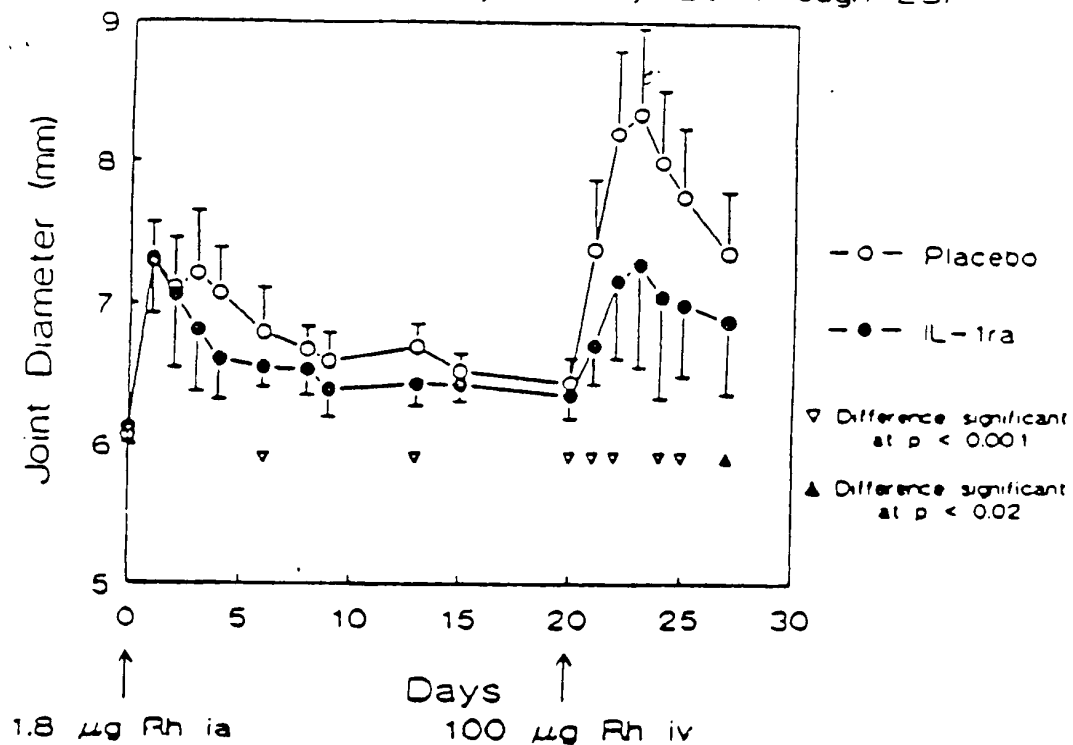
Figure 4



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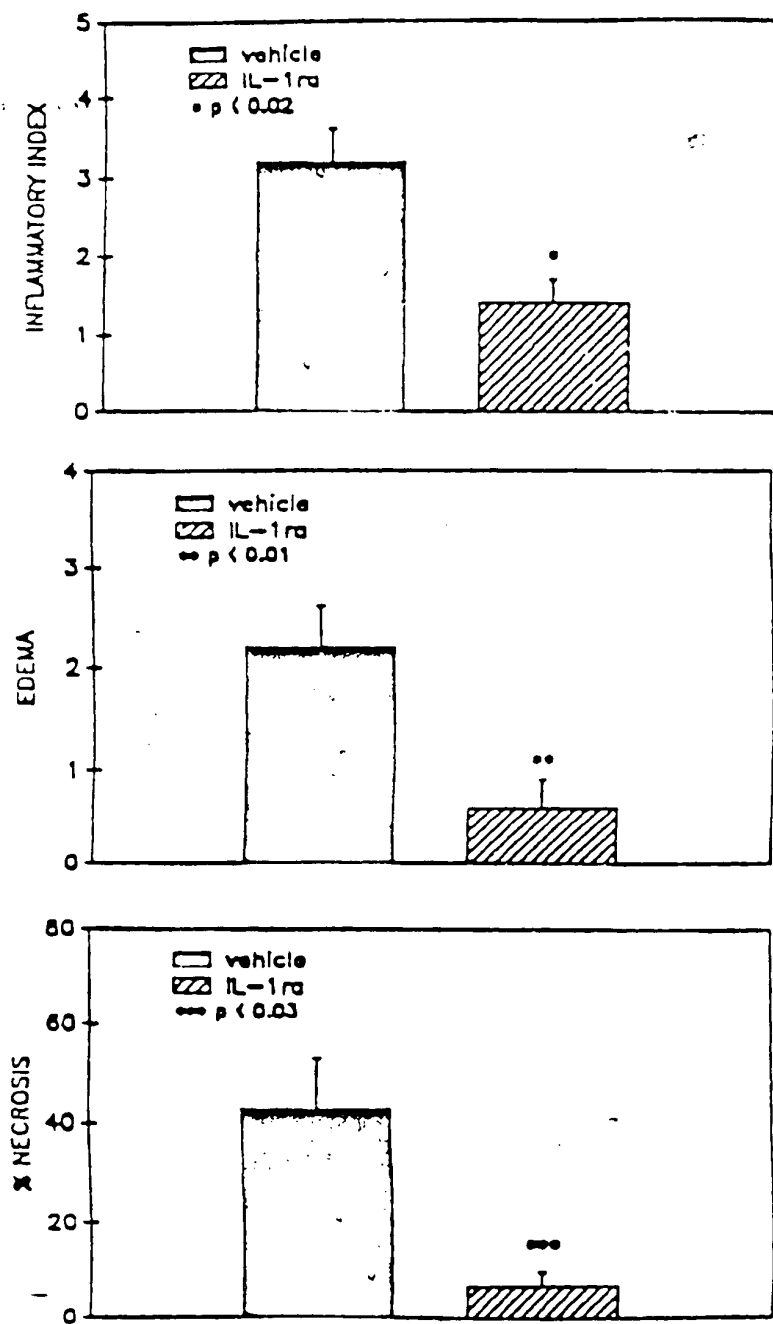
Figure 5

Effects of IL-1ra on SCW reactivation of joint inflammation
(1 mg/kg 4 times daily on day 20 through 23)



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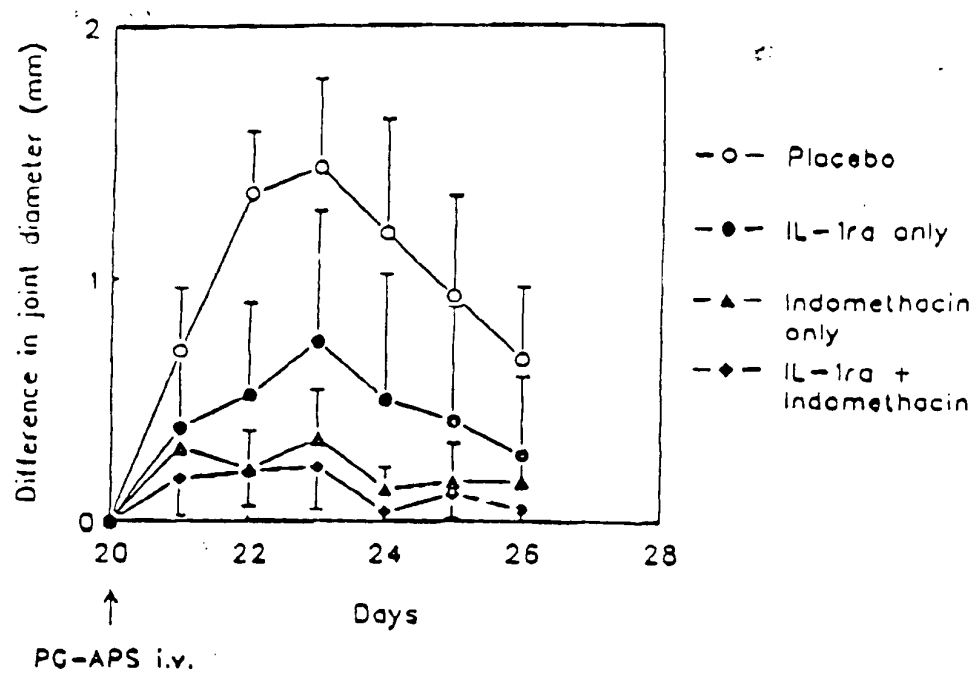
FIG. 6



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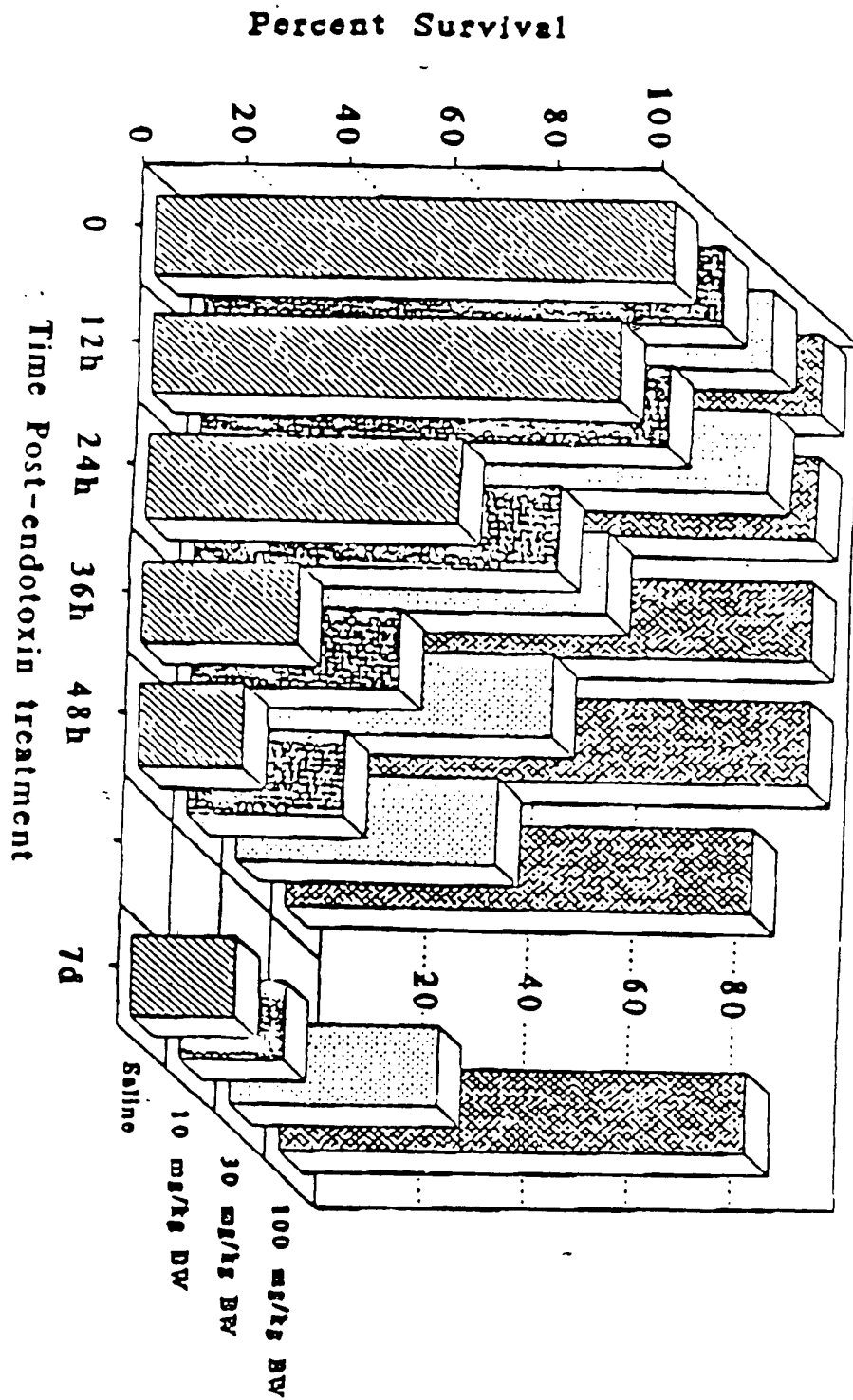
Figure 7

Effects of IL-1ra on PG-APS reactivation of joint inflammation



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Figure 8



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